PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

14

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
UCL-005-PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/04632	22/05/2000	11/08/1999
International Patent Classification (IPC) or national classification and IPC C07B59/00		
Applicant UNIVERSITE CATHOLIQUE DE LOUVAIN		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 1 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
02/03/2001	15.11.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Butkowskyj-Walkiw, T Telephone No. +49 89 2399 8594

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/04632

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-6,8-17	as originally filed			
7	as received on	19/10/2001	with letter of	15/10/2001

Claims, No.:

1-30	as originally filed

Drawings, sheets:

1/7-7/7	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 26-30.

because:

   ☒ the said international application, or the said claims Nos. 26-30 with respect to industrial applicability relate to the following subject matter which does not require an international preliminary examination (*specify*):
   see separate sheet

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-9,11-30
	No:	Claims	10
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-30
Industrial applicability (IA)	Yes:	Claims	1-25
	No:	Claims	26-30

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

III.

Claims 26-30 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

V.

D1 (Olivier Josse et al; Synthesis, pages 404 and 405, composition 3) is novelty destroying (Art. 33(2) PCT) for the present claim 10.
In the light of the closest prior art document D2 (WO-A-94/11348) the present claims 1-9,11-30 have to be considered as being novel as said citation does not explicitly disclose the present subject-matter.

Further, the present claims 1-30 are open to objection as to lack of inventive step (Art. 33(3) PCT) as the object of the present application, namely to provide a method for synthesizing perfluorinated radiolabelled bioactive compounds which selectively react with a target present in patient cells, and the presently claimed solution, has already been suggested by D2 (page 12, lines 18,19; example 1; claims 1-43).

For the assessment of the present claims 26-30 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

VII.

The Applicant's attention is drawn to the fact that claims shall not rely in respect of the technical features of the invention, on references to the figures (Rule 6.2a) PCT).

VIII.

The expression "synthetically equivalent group" in claim 10 is unclear (Art. 6 PCT).

a) adding a THF solution of 2 of Figure 7 to a suspension of PYBOP in THF followed by Et_3N,

b) adding an amine 1 of Figure 7 and Et_3N to the solution obtained in step (a),

c) adding a catalytic amount to the solution obtained in step (b) of pTsOH and refluxing the solution,

d) cooling the solution obtained after step (c) at ambient temperature and adding a sodium bicarbonate solution,

e) extracting the product obtained after step (d) with ethyl acetate and drying and concentrating the product with ethyl acetate,

f) purifying the residue obtained after step (e) by column chromatography on silica gel,

g) removing traces of water by washing the product of step (f) with trifluoroacetic anhydride,

h) reacting said persulphurated derivative obtained from step (g) with a suitable labelled or non-labelled perfluorinating agent and a suitable oxidant resulting in a compound having a high yield of fluor atom incorporation,

i) deprotecting the nitrogen function, resulting in a perfluoroalkyl amine derivative, and

j) coupling the perfluoroalkyl amine derivative obtained in step (i) with an activated form of 2-(2-nitro-imidazol-1-yl) acetic acid, resulting in the [^{18}F]-labelled or non-labelled perfluorinated-nitroaromatic compound.

Compound 3 as shown in Figure 7 is designated as Ethyl-3-(N-phthalimido)aminopropanedithioate. Compound 1 and its synthesis have been described in Josse et al., 1999 (10). Preferably, said method is used to prepare namely N-(phthalimido)3,3,3-trifluoropropylamine having the general formula of the endproduct 4 of the reaction scheme presented in Figure 8 as described in Example 2.

Said first intermediate perfluorinated compound according to this embodiment of the present invention is used for the synthesis of a second

PATENT COOPERATION TREATY

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INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
UCL-005-PCT		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 00/ 04632	22/05/2000	11/08/1999
Applicant		
UNIVERSITE CATHOLIQUE DE LOUVAIN		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
[redacted]EP 00/04632

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07B59/00 C07D209/48 C07C211/03 G01N33/58

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07B C07D C07C G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
BEILSTEIN Data, CHEM ABS Data, WPI Data, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	J. B. DICKEY ET AL.: "Fluorinated Anthraquinone Dyes" INDUSTRIAL AND ENGENEERING CHEMISTRY, vol. 48, 1956, pages 209-213, XP000874162 WASHINGTON, US page 212, column 2, line 17 - line 37 ---	10
A	WO 94 11348 A (THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA) 26 May 1994 (1994-05-26) cited in the application page 212, column 2, line 17; claims; example 1 --- -/--	1,10, 21-30

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 September 2000	22/09/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Zervas, B

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	OLIVIER JOSSE ET AL.: "A Convenient Synthesis of Ethyl 3-Aminopropanedithioate (beta-Alanine Ethyl Dithioester)" SYNTHESIS, March 1999 (1999-03), pages 404-406, XP002129104 STUTTGART DE cited in the application page 404, column 2; claims; example 1 page 405, column 2, line 7 - line 13; example 1 ---	10
A	WO 95 09844 A (BOARD OF RAGENTS, THE UNIVERSITY OF TEXAS SYSTEM) 13 April 1995 (1995-04-13) page 405, column 2, line 7; claims; examples -----	1,10, 21-30

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
EP 00/04632

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9411348 A	26-05-1994	CA 2149770 A	26-05-1994
		EP 0669913 A	06-09-1995
		JP 8503469 T	16-04-1996
		US 5540908 A	30-07-1996
		US 5843404 A	01-12-1998
WO 9509844 A	13-04-1995	AU 8074294 A	01-05-1995
		US 5728843 A	17-03-1998
		US 5886190 A	23-03-1999

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 28 May 2001 (28.05.01)	
International application No. PCT/EP00/04632	**Applicant's or agent's file reference** UCL-005-PCT
International filing date (day/month/year) 22 May 2000 (22.05.00)	**Priority date** (day/month/year) 11 August 1999 (11.08.99)
Applicant MARCHAND, Jacqueline et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

02 March 2001 (02.03.01)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Charlotte ENGER

Telephone No.: (41-22) 338.83.38

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

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22 February 2001 (22.02.2001)

PCT

(10) International Publication Number
WO 01/12575 A1

(51) International Patent Classification[7]: C07B 59/00, C07D 209/48, C07C 211/03, G01N 33/58

(21) International Application Number: PCT/EP00/04632

(22) International Filing Date: 22 May 2000 (22.05.2000)

(25) Filing Language: English

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(30) Priority Data:
99870172.6 11 August 1999 (11.08.1999) EP

(71) Applicant *(for all designated States except US)*: **UNIVERSITE CATHOLIQUE DE LOUVAIN** [BE/BE]; Place De L'Université 1, B-1348 Louvain-la-Neuve (BE).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **MARCHAND, Jacqueline** [BE/BE]; Rue Du Basson 48, B-6001 Marcinelle (BE). **GREGOIRE, Vincent** [BE/BE]; Rue Du Fond Marie Monseu 17, B-1330 Rixensart (BE).

(74) Agent: **BRANTS, Johan, Philippe, Emile**; De Clercq, Brants & Partners cv, E. Gevaertdreef 10 A, B-9830 Sint-Martens-Latem (BE).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/12575 A1

(54) Title: METHODS FOR PREPARING PERFLUORINATED [^{18}F]-RADIOLABELLED NITROIMIDAZOLE DERIVATIVES FOR CELLULAR HYPOXIA DETECTION

(57) Abstract: The present invention relates to chemical synthesis of radiolabelled perfluorinated bioactive compounds. More particularly, the present invention relates to radiolabelled compounds to be used as indicators for tissue hypoxia. More particularly, the present invention relates to the synthesis and the use of [^{18}F] labelled perfluorinated nitroimidazole compounds having an incorporation of [^{18}F] atoms characterized by a specific radioactivity of the compound comprised between 1 and 30 Ci/mmol, preferably between 1 and 20 Ci/mmol, preferably 1 and 10 Ci/mmol. More particularly to [^{18}F] labelled EF3 or [^{18}F] labelled EF5. The present invention also relates to a method for the detection of tissue hypoxia in a patient comprising introducing an [^{18}F] labelled nitroimidazole compound into said patient, imaging tissue hypoxia in said patient, and, quantifying tissue hypoxia in said patient.

METHODS FOR PREPARING PERFLUORINATED [^{18}F]-RADIOLABELLED NITROIMIDAZOLE DERIVATIVES FOR CELLULAR HYPOXIA DETECTION

The present invention relates to the field of chemical synthesis of radiolabelled bioactive compounds for the detection of specific targets present in the tissues or cells of a patient and to the obtained [^{18}F]-radiolabelled compounds and to the intermediates used in the method for their preparation.

In a first aspect the invention is related to [^{18}F]-labelled perfluorinated compounds, in particular [^{18}F]-EF3 and [^{18}F]-EF5.

In a second aspect the invention is related to a method for the preparation of said labelled compounds and to their intermediates.

In a third aspect the invention is also related to the use of said [^{18}F]-labelled compounds for cellular hypoxia detection.

More particularly, the present invention relates to the field of chemical synthesis of radiolabelled compounds to be used as indicators of tissue hypoxia. The present invention provides methods for preparing said type of compounds, as well as useful precursors in said synthesis and methods for preparing the same. The present invention also relates to methods of using these radiolabelled bioactive compounds for the detection of specific targets present in tissues of a patient. More particularly the present invention relates to the detection of tissue hypoxia in a patient using said radiolabelled compounds.

Cellular hypoxia is a typical feature in various physiopathological processes as frequent as malignant tumor development, heart disease, stroke, diabetes and wound healing. In malignant tumors, experimental and clinical evidences have shown that the hypoxic fraction may influence the malignant phenotype, the growth rate and may reduce the sensivity to ionizing radiation and chemotherapeutic agents. In head and neck lymph nodes, and in cervix carcinomas for example, tumour hypoxia is associated on an individual basis with a higher rate of local recurrence after radiotherapy. In stroke and heart infarct, it has been shown that

the severity of the tissue function impairment critically depends on the location and the amount of ischemic tissue.

In this framework, accurate determination of the magnitude of tissue hypoxia has always been a focus of intensive research. Such information is of great value as a prognostic factor of the severity of the disease, and as a tool to select for alternative therapies and to monitor the response to therapeutic interventions. In oncology, recent development in microelectrode techniques has permitted to measure the oxygen partial pressure (PO_2) in experimental as well as clinical tumors. This technique has greatly contributed to the current knowledge on the influence of hypoxia in tumor physiopathology and response to treatment. Such a technique however has important limitations. The sensitivity of the method is far from being optimal in the range of PO_2 values (<10 mm Hg) of interest in oncology. It lacks specificity as it is also very much influenced by the amount of tissue necrosis around the microelectrode. Besides, it is an invasive and time consuming technique that will never be spread out in a routine clinical environment. In stroke and heart disease, there is no method to directly measure tissue hypoxia which can thus only be inferred from indirect measurements of tissue metabolism and vascularisation.

Tumor hypoxia is detected using hypoxia-binding chemical markers. These markers are nitroheterocyclic compounds which exhibit a particular metabolism under hypoxic cellular conditions, and hence can covalently bind to intracellular macromolecules (e.g. proteins, RNA, lipids and DNA). These reduced moieties trapped into hypoxic cells, can be detected by immunofluorescence on tissue section or by flow cytometry using for both techniques specific antibodies. Tagged with an appropriate radioactive isotope, these reduced moieties could also be detected by nuclear medicine techniques. Misonidazole is the prototype of hypoxia-binding chemical markers. More recently, a tri- and pentafluorinated nitroimidazole derivates, designated EF3 and EF5, respectively, have been synthesized (US patent No. 5,540,908 in name of Koch). In comparison with misonidazole, these 2 compounds have several advantages. Both compounds

have a more specific binding to hypoxic cells, and the binding does not depend on the intracellular level of reductase systems. In addition, fluorochrome-conjugated specific antibodies have been generated for both EF3 and EF5. Oxygen-dependent binding have been reported in various experimental systems such as EMT6 spheroids, EMT6 tumors and Moris 7777 rat tumors. EF5 has been very recently approved by American Authorities for human studies and a phase I trial is in progress in the USA. Although very sensitive and specific, determination of cellular hypoxia with EF5 or EF3 remains however invasive as its requires the use of tissue specimens.

The [^{18}F] monofluorination of bioactive compounds is known; usually, the syntheses make use of the classical nucleophilic displacement of a leaving group with [^{18}F] fluoride anion. In particular, the method has been applied to the preparation of [^{18}F]-fluoroethanidazole (1) and [^{18}F]-fluoromisonidazole (2), and [^{18}F]-fluoroerythronitromidazole (3), three members of the nitroimidazole family.

In organic synthesis, the direct and selective perfluorination (- CF_2, - CF_3 and - C_2F_5) remains a difficult problem because the classical nucleophilic substitution strategy could not be efficiently applied (4). The preparation of perfluorinated molecules is increasingly problematical by way of simple fluorination techniques as the number of fluorine included in molecules increases; this results from strong electronic repulsions between the fluorine atoms already present in a given molecule and the fluoride reagent which would enter into the molecule. Therefore, the construction of a CF_3 group, from a carboxylic precursor, is usually carried out by the very strong and toxic reagent SF_4 (4). Recently, an alternative solution has been brought, making use of sulphurated precursors (ortho-trithioesters and dithioesters), halonium ions and an HF-reagent; however, this method is only applicable in the case of poorly functionalized aromatic compounds and conjugated compounds (5). In the aliphatic series, one precedent has been found, concerning the introduction of a CF_2 group into non-functionalized alkyl chains (6).

Concerning the [^{18}F]-labelling of a CF_3 group, the substitution method from a CF_2Br precursor has been used for two specific applications in which the competition with an elimination reaction could not occur (7). The [^{18}F]-labelling of CF_2 and CF_3 group from non-functionalized aromatic and aliphatic persulphurated precursors has been recently described by our group (8).

As far as functionalized aliphatic precursors are concerned (for instance, aminoacid derivatives), no precedent has been found in the literature for the [^{18}F]-labelling of CF_2,CF_3 or C_2F_5 groups; this is the subject of the present invention.

The present invention aims at developing and testing labelled bioactive compounds, more particularly [^{18}F]-EF3 and [^{18}F]-EF5 for *in vivo* detection of hypoxia. Such a method would permit measurements of both the hypoxic fraction and the distribution of hypoxia within an individual tissue or tumor. In comparison with the existing methods for measuring hypoxia (e.g., microelectrode, immunofluorescence and/or flow cytometry to detect hypoxia-binding chemical markers), the PET detection is a non-invasive technique that would allow individual measurements in any tumors and tissues. In comparison with other nuclear medicine techniques (e.g. SPECT), the PET camera detection offers the advantage of a better spatial resolution and a much more accurate quantification of the radioactivity. In comparison with other hypoxia-binding chemical markers, [^{18}F]-EF3 and [^{18}F]-EF5 would maintain both their superior specificity and sensitivity for hypoxic cells as observed for the unlabelled parent compounds. Such a technique could be easily combined with anatomic imaging modalities (e.g. CT Scanner and MRI) allowing a better mapping of the distribution of hypoxia in a specific tissue/organ. In addition, the detection of hypoxia by the PET method could also be combined with other functional imaging techniques (e.g. fMRI, PET with other markers) investigating important physiological parameters such as tissue proliferation or metabolism. Such a combined approach should allow to non-invasively study intriguing physiopathological questions related to tumor

development and response to treatment, or to functional tissue defect after an ischemic injury. Important physiopathological questions related to tumor development and response to treatment, or to the understanding of functional tissue defect after an ischemic injury could be investigated by this nuclear medicine technique.

Thus assessment of tissue hypoxia with [^{18}F]-EF3 or [^{18}F]-EF5 is likely to allow significant benefits to the management of cancer and other human diseases. The structures of EF3 and EF5 are shown in Figure 1 (9).

The present invention thus aims at methods for synthesizing perfluorinated radiolabelled bioactive compounds which selectively react with a target present in patient cells.

The present invention concerns the preparation of original sulphur-containing precursors as a first intermediate allowing the direct radiolabelling of perfluoroalkyl groups (-CF_3, -CF_2-) by [^{18}F] on substrates equipped with nitrogen-containing functions. This first intermediate is defined in claims 10, 11, 12 and 13. The present invention also concerns the [^{18}F]-labelled perfluorinated second intermediate as defined in claims 14, 15, 16 and 17 and the [^{18}F]-labelled perfluorinated third and last intermediate having the formula of a perfluoropropylamine as defined in claims 18, 19 and 20.

More particularly, the present invention aims at methods for synthesizing [^{18}F] labelled perfluorinated nitroimidazole derivatives, more particularly methods for synthesizing [^{18}F] labelled EF3 and EF5.

The present invention also aims at useful sulphur-containing precursors for synthesizing said compounds and methods for preparing the same.

The present invention further relates to the different uses of said perfluorinated radiolabelled bioactive compounds, more particularly the different uses of [^{18}F] labelled nitroimidazole derivatives, and even more particularly the uses of [^{18}F] labelled EF3 and EF5.

According to a first aspect the invention relates to novel [^{18}F]-radiolabelled compounds as worded in claims 1-4.

According to another aspect, the present invention relates to a first type of precursor compound which is an amino acid derivative which is N-protected by an imido group or a synthetically equivalent group and wherein the carboxyl function has been transformed into a dithioester function or a synthetically equivalent persulphurated moiety.

Such persulphurated amino acid derivatives may be used to prepare perfluorinated alkylamine derivatives using a suitable perfluorinating agent and a suitable oxidant as described below.

Said persulphurated compound may be derived from any of the following amino acids: α-amino acids of the natural pool such as: glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, asparagine, glutamine, aspartate, glutamate, and bis-protected serine threonine, lysine, arginine, histidine; α-amino acids of synthetic origin and derivatives thereof; β-amino acids such a 3-aminopropionic acid and derivatives thereof, γ-amino acids such as 4-aminobutyric acid and derivatives thereof, δ-amino acids such as 5-aminovaleric acid and derivatives thereof, ε-amino acids such as 6-aminocaproic and derivatives thereof and similarly, the ω-amino acid derivatives.

Said imido group may be any imido group known in the art, such as for instance a phthalimido group.

Said first type of precursor compound is used for the synthesis of a second and third precursor compound as described below. Figure 2 in combination with Figure 4 describes a representative synthesis of a precursor derived from β-alanine. Ethyl 3-(N-phthalimido)aminopropanedithioate having the general formula of the endproduct (3) of the reaction scheme presented in Figure 7 or Figure 2 is an example of a persulphurated beta-alanine derivative.

According to a preferred embodiment, the present invention relates to a method for preparing a compound of claims 1-4 as shown in Figure 7 and 8 comprising the following steps:

a) adding a THF solution of 2 of Figure 7 to a suspension of PYBOP in THF followed by Et_3N,

b) adding an amine 1 of Figure 7 and Et_3N to the solution obtained in step (a),

c) adding a catalytic amount to the solution obtained in step (b) of pTsOH and refluxing the solution,

d) cooling the solution obtained after step (c) at ambient temperature and adding a sodium bicarbonate solution,

e) extracting the product obtained after step (d) with ethyl acetate and drying and concentrating the product with ethyl acetate,

f) purifying the residue obtained after step (e) by column chromatography on silica gel,

g) removing traces of water by washing the product of step (f) with trifluoroacetic anhydride,

h) reacting said persulphurated derivative obtained from step (g) with a suitable labelled or non-labelled perfluorinating agent and a suitable oxidant resulting in a compound having a high yield of fluor atom incorporation,

i) deprotecting the nitrogen function, resulting in a perfluoroalkyl amine derivative, and

j) coupling the perfluoroalkyl amine derivative obtained in step (i) with an activated form of 2-(2-nitro-imidazol-1-yl) acetic acid, resulting in the [^{18}F]-labelled or non-labelled perfluorinated-nitroaromatic compound.

Compound 3 as shown in Figure 7 is known as Ethyl-3-(N-phthalimido)aminopropanedithioate. Compound 1 and its synthesis have been described in Josse et al., 1999 (10). Preferably, said method is used to prepare namely N-(phthalimido)3,3,3-trifluoropropylamine having the general formula of the endproduct 4 of the reaction scheme presented in Figure 8 as described in Example 2.

Said first intermediate perfluorinated compound according to this embodiment of the present invention is used for the synthesis of a second

intermediate compound which is an amino synthon which can be incorporated in the synthesis of [^{18}F] labelled target-bioactive compounds by using the classical methods of peptide coupling, or other coupling methods. Examples of suitable perfluorinating agents and suitable oxidants according to the present invention are given in Figure 3. Details of the preparation process of the [^{18}F] perfluorating agents appear in the earlier publications (8).

According to a preferred embodiment, the present invention relates to a perfluorinated derivative compound obtainable by a reaction wherein hydrogen fluoride/pyridine complex (HF-Pyridine) is used as a perfluorinating agent and 1,3-dibromo-5,5-dimethylhydantoin (DBH) is used as an oxidant, resulting in a compound having a high yield of fluor atom incorporation. Said perfluorinated reagent and reaction product can contain [^{19}F] or [^{18}F].

According to another embodiment, the present invention also relates to mixtures of [^{19}F] and [^{18}F] labelled perfluorinated derivative compounds as defined above.

The [^{18}F] isotope is incorporated in such an amount that the specific radioactivity of the compound is comprised between 1 and 30 Ci/mmol, preferably between 1 and 20 Ci/mmol, preferably between 1 and 10 Ci/mmol.

According to an even more preferred embodiment, the present invention relates to a compound having the formula of the endproduct 4 of the reaction as shown in Figure 8.

According to a preferred embodiment, the present invention relates to a perfluorinated derivative of the compound of claims 1-4 obtainable according to a reaction defined in any of claims 5-8.

According to another embodiment, the present invention relates to a final precursor compound which can be incorporated into the synthesis of [^{18}F] target-bioactive compounds and which is a perfluorinated derivative of the first precursor described above wherein the nitrogen function has been deprotected by refluxing into hydrazine solution, or by using other deprotection methods, resulting in a perfluoroalkyl amine derivative.

According to another embodiment, the present invention relates to a mixture of the radiolabelled perfluorinated bioactive compound and the non-radioactive labelled bioactive compound as defined above.

Preferably said final precursor according to the present invention is described in Figure 4 and is the endproduct (5). Since no procedure presently exists for direct and selective perfluorination of N-functionalized aliphatic compounds, the present invention brings a significant advance in organic chemistry in general (unlabelled compounds), and in the [^{18}F] radiolabelling of biologically active compounds in particular.

The method according to the present invention is flexible since [^{18}F]-perfluorinated alkylamines can be used as building blocks in various total syntheses of pharmaceuticals. The method illustrated in the examples section can easily be expected to be extended to any other amino acid of interest.

According to a preferred embodiment, the present invention thus relates to an [^{18}F] labelled bioactive compound synthesized using as a precursor a perfluorinated derivative.

According to a preferred embodiment, the present invention thus relates to the use of said perfluorinated derivative having the formula of the endproduct 5 of the reaction scheme as shown in Figure 4 for chemical synthesis of an [^{18}F] labelled perfluorinated nitroimidazole having an incorporation of [^{18}F] atoms in such an amount that the specific radioactivity of the compound is comprised between 1 and 10 Ci/mmol.

According to a preferred embodiment, said [^{18}F] labelled perfluorinated nitroimidazole compound is [^{18}F] labelled EF3 having a general formula as set out in Figure 1.

According to another preferred embodiment, said [^{18}F] labelled perfluorinated nitroimidazole compound is [^{18}F] labelled EF5 having a general formula as set out in Figure 1. [^{18}F] labelled EF5 can be prepared by using an appropriate persulphurated precursor (see Figure 6 for potential precursor types).

The present invention also relates to a method for the detection of tissue hypoxia in a patient comprising:

introducing an [^{18}F] labelled nitroimidazole compound as defined above into said patient,

imaging tissue hypoxia in said patient, and,

quantifying tissue hypoxia in said patient.

Said patient is preferably a mammal and more preferably a human. Preferred nitroimidazole compounds to be used according to this embodiment of the invention are [^{18}F] labelled EF3 or [^{18}F] labelled EF5.

Methods for detecting tissue hypoxia in patient tissue include, but are not limited to non-invasive imaging techniques, immunohistochemistry, immunofluorescence, autoradiography and flow cytometry. Imaging techniques include, but are not limited to positron emission tomography (PET). Generally, imaging techniques involve administering a compound with marker atoms which can be detected externally to the mammal. A compound of the invention is dissolved or dispersed in a pharmaceutically acceptable diluent, such as non-pyrogenic physiological saline, is administered to the patient preferably intravenously. After administration, time is allowed for metabolisation (reduction) of the hypoxic marker and clearance of the non-metabolized compound. Tissue hypoxia is then assayed using one or several of the methods described above. Non-invasive imaging techniques can indeed be combined with immunohistochemistry, immunofluorescence, autoradiography or flow cytometry on tissue specimen.

According to a preferred embodiment, the detection technique used in said method is positron emission tomography.

The present invention also relates to a method for the detection of tissue hypoxia in a tissue comprising:

introducing an [^{18}F] labelled nitroimidazole compound as defined above into a patient,

removing a tissue sample from said patient, and,

analysing the emission in said tissue sample by autoradiography.

Said patient is preferably a mammal and more preferably a human. Preferred nitroimidazole compounds to be used according to this embodiment of the invention are [^{18}F] labelled EF3 or [^{18}F] labelled EF5.

Also here, a compound of the invention, is dissolved or dispersed in a pharmaceutically acceptable diluent, such as non-pyrogenic physiological saline, is administered to the patient preferably intravenously. After administration time is allowed for metabolisation (reduction) of the hypoxic marker and clearance of the non-metabolized compound. A sample of for instance tumor tissue taken from the patient is then analyzed. Methods of obtaining tissue samples include any surgical and non-surgical technique known in the art. Surgical methods include, but are not limited to biopsy such as fine needle aspirate, core biopsy, dilation and curettage.

According to another embodiment, the present invention relates to a method for the detection of [^{18}F] labelled bioactive compound in a patient comprising:

a) introducing an [^{18}F] labelled bioactive compound according to claims 1-4 into said patient,

b) imaging the presence of said [^{18}F] labelled bioactive compound in said patient,

c) quantifying the presence of said [^{18}F] labelled bioactive compound in said patient.

Alternatively, the present invention also relates to a method for the detection of [^{18}F] labelled bioactive compound in a tissue comprising:

a) introducing an [^{18}F] labelled perfluorinated nitroimidazole compound as defined above into a patient,

b) taking a tissue sample from said patient, and,

c) analysing the emission in said tissue sample by autoradiography.

Said patient is preferably a mammal and more preferably a human.

Preferred nitroimidazole compounds to be used according to this embodiment of the invention are [^{18}F] labelled EF3 or [^{18}F] labelled EF5.

The examples as set out below are purely illustrative of a representative synthesis according to the embodiments of the present invention and are by no way intended to limit the present invention as set out in detail above. The content of all references referred to in this text is incorporated by reference.

ABBREVIATIONS

EEDQ	N-Ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline
PYBOP	Benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium-hexafluorophosphate
THF	tetrahydrofuran
pTsOH	para-toluenesulfonic acid
Et_3N	triethylamine
DBH	dibromodimethylhydantoin
PET	Positron Emission Tomography
MRI	Magnetic Resonance Imaging
fMRI	functional Magnetic Resonance Imaging
SPECT	Single Photon Emission Computed Tomography
TLC	Thin layer chromatography

FIGURE LEGENDS

Figure 1 describes the chemical structure of EF5 and EF3

Figure 2 describes a reaction scheme for the synthesis of beta-Alanine Ethyl Dithioester. A part of this reaction scheme was previously described in Josse et al., 1999 (10).

Figure 3 describes the structure of the different perfluorinating agents and oxidants to be used in the labelling process according to the present invention.

Figure 4 describes the reaction scheme to prepare an [^{18}F]-perfluoroalkyl amine derivative from the persulphurated first precursor obtained in Figure 2.

Figure 5 describes the synthesis of EF3. Radiolabelled EF3 is made by using [^{18}F] perfluoroalkyl amine derivate of Figure 4 as a precursor in the last reaction step. X = 2,3,5,6-tetrafluorophenoxy.

Figure 6 describes the different possible potential precursor types for the synthesis of EF5.

Figure 7 describes the reaction scheme for the synthesis of ethyl 3-(N-phthalimido)aminopropanedithioate as described in Example 1

Figure 8 describes the reaction scheme for the synthesis of N-(phthalimido)3,3,3-trifluoropropylamine as described in Example 2.

EXAMPLES

Example 1: Synthesis of ethyl 3-(N-phthalimido)aminopropanedithioate

A THF solution of 2 is added to a suspension of PYBOP in THF followed by Et_3N; the mixture is stirred during 40 minutes at 20°C. Then, amine 1 (as the trifluoroacetate salt; (10) and Et_3N are added, and the mixture is stirred during 3 h at 20°C. After this reaction time and the addition of a catalytic amount of pTsOH, the solution is refluxed overnight. After cooling at ambient temperature, a sodium bicarbonate solution is added and the product is extracted with ethyl acetate. Drying ($MgSO_4$) and concentration under reduced pressure gave crude 3. The residue is purified by column chromatography on silica gel (hexane/ethyl acetate 30:70). Last traces of water are removed by washing the product with trifluoroacetic anhydride. The total yield was 85%. A yellow solid product was obtained. Spectral data : ^1H NMR ($CDCl_3$, 200 MHz) δ 1.26 (t, 3H, *J*=7.3 Hz), 3.18 (q, 2H, *J*= 7.3 Hz), 3.37 (t, 2H, *J*=7 Hz), 4.14 (t, 2H, *J*= 7 Hz), 7.73 (m, 2H), 7.85 (m, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz) ppm 11.93, 30.70, 38.24, 49.13, 123.30, 131.99, 167.97, 233.00.

Example 2: Synthesis of [^{18}F]-labelled N-(phthalimido)3,3,3-trifluoropropylamine

The [^{18}O]-water solution of [^{18}F]-hydrogen fluoride is neutralized by a small amount of aqueous potassium hydroxide. Water is removed by evaporation till dryness, under an argon flux to give the potassium salt ([^{18}F]-KF). Then, addition of a first portion of a dichloromethane solution of HF-Pyridine provides the desired radiolabelling agent. DBH is added and the mixture cooled to –78°C before introducing 3. The solution is allowed to reach the ambient temperature and is stirred for 30 minutes. A second fraction of a dichloromethane solution of HF-Pyridine is added for completing the reaction within 30 minutes. The trifluoromethyl amine 4 is recovered with a specific radioactivity comprised between 1 and 30

Ci/mmol, as measured by Radio-TLC (Radio-Thin Layer Chromatography). ^{19}F-NMR (282 MHz) δ - 66.2 (t, J = 10.5 Hz).

Example 3: Synthesis of [^{18}F]-labelled 3,3,3-trifluropropylamine

N-(Phthalimido) 3,3,3-trifluoropropylamine 4 is dissolved in acetonitrile and hydrazine hydrate (2 : 1), and heated at 75°C. The free amine is distilled under a slow stream of argon. The product is identified by comparison of the retention time in gas chromatography with authentic material.

Example 4: Synthesis of [^{18}F]-EF3

The [^{18}F]-3,3,3-trifluoropropylamine, prepared from 15 mg of ethyl 3-(N-phthalimido) aminopropanedithioate, was distilled and condensed at 0°C in an acetonitrile solution of 2,3,5,6-tetrafluorophenyl 2-(2-nitro-imidazol-1-yl) acetate obtained according to Tewson (1) (30 mg/3 ml of CH_3CN). The mixture was stirred for 30 min. at 20°C, then purified by chromatography on silica gel with ethyl acetate as eluent. [^{18}F]-EF3 was recovered in 63 % yield as assayed by radio - TLC.

REFERENCES

(1) Tewson, T.J. *Nuclear Medicine and biology* **1997**, *24* (8), 755.

(2) Rasey, J.S. et al., *Radiation Research* **1987**, *111*(2), 292 and *Internat. J. Radiation Oncology, Biology, Physics* **1996**, *36* (2), 417; Grierson, J.R. et al. *J. Nuclear Med.* **1989**, *30* (3), 343; Koh, W.J. et al. *Internat. J. Radiation Oncology, Biology, Physics***1992**, *22* (1), 199.

(3) Yang, D.J. et al. *Radiology* **1995**, *194* (3), 795;Cherif, A. et al. *US 5*, 886, 190 (1999).

(4) Kitazume, T. and Yamazaki, T. "Experimental Methods in Organic Fluorine Chemistry", Gordon and Breach Science Publishers, Kodansha(Tokyo), **1998**.

(5) Kuroboshi, M. and Hiyama, T. *Chem. Letters* **1992**, 827 and *Synlett* **1991**, 909; Furuta, S. and Hiyama, T. *Synlett* **1996**, 1199; Matthews, D.P. et al. *Tetrahedron Letters* **1986**, *27*, 4861.

(6) Sondej, S.C. and Katzenellenbogen, J.A. *J. Org. Chem.* **1986**, *51*, 3508.

(7) Johnström, P. et al. *J. Labelled Cpd Radiopharm.* **1995**,*36*, 537 and *Appl. Rad. Isotopes* **1996**, *47*, 401.

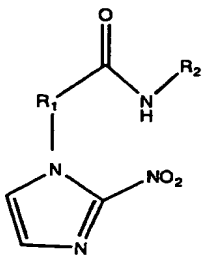
(8) Josse, O. et al. *J. Labelled Cpd Radiopharm.* **1998**, *40*, 48 and *J. Labelled Cpd Radiopharm.* **2000**, *42*,315.

(9) Koch, C.J. *US 5*, 540, 908 (1996); Koch, C.J. et al. *British J. Cancer* **1995**, *72* (4), 869; Baird, I.R. et al. *Synth. Commun.* **1998**, *28*, 3701.

(10) Josse, O.; Labar, D.; Marchand-Brynaert, J. *Synthesis* **1999**, 404.

CLAIMS

1. A [^{18}F]-labelled perfluorinated-nitroaromatic compound having the formula:



wherein R_1 is CH_2 and R_2 is an alkyl group having up to about 6 halogen atoms, wherein said alkyl group has the formula $CHXCX_2$ CY_3 where X is halogen or hydrogen and Y is fluorine.

2. A compound according to claim 1 having specific radioactivity of the compound comprised between 1 and 30 Ci/mmol, preferably between 1 and 20 Ci/mmol, preferably between 1 and 10 Ci/mmol.

3. A compound according to claim 1 or 2 having the formula 2-(2-nitro-1H-imidazol-1-yl)-N-(3,3,3-trifluoropropyl) acetamide ([^{18}F]-EF3).

4. A compound according to claim 1 or 2 having the formula 2(2-nitro-1H-imidazol-1-yl)-N-(2,2,3,3,3-pentafluoropropyl) acetamide ([^{18}F]-EF5).

5. A method for the synthesis of a compound according to one of the claims 1-4, comprising the step of coupling 2-(2-nitro-imidazol-1-yl) acetic acid with a [^{18}F]-labelled perfluoroalkyl amine derivative.

6. A method according to claim 5, wherein said coupling is a classical peptide coupling using a derivative of 2-(2-nitro-imidazol-1-yl) acetic acid in which the OH group of the carboxyl function has been replaced by a good leaving group.

7. A method for the synthesis of a compound according to of one of the claims 1-4 or the corresponding non-labelled form thereof, comprising the steps of:

a) adding a THF solution of 2 of Figure 7 to a suspension of PYBOP in THF followed by Et_3N,

b) adding an amine 1 of Figure 7 and Et_3N to the solution obtained in step (a),

c) adding a catalytic amount to the solution obtained in step (b) of pTsOH and refluxing the solution,

d) cooling the solution obtained after step (c) at ambient temperature and adding a sodium bicarbonate solution,

e) extracting the product obtained after step (d) with ethyl acetate and drying and concentrating the product with ethyl acetate,

f) purifying the residue obtained after step (e) by column chromatography on silica gel,

g) removing traces of water by washing the product of step (f) with trifluoroacetic anhydride,

h) reacting said persulphurated derivative obtained from step (g) with a suitable labelled or non-labelled perfluorinating agent and a suitable oxidant resulting in a compound having a high yield of fluor atom incorporation,

i) deprotecting the nitrogen function, resulting in a perfluoroalkyl amine derivative, and

j) coupling the perfluoroalkyl amine derivative obtained in step (i) with an activated form of 2-(2-nitro-imidazol-1-yl) acetic acid, resulting in the [^{18}F]-labelled or non-labelled perfluorinated-nitroaromatic compound.

8. A method according to claim 7 wherein hydrogen fluoride/pyridine complex (HF-Pyridine) is used as a perfluorinating agent and 1,3-dibromo-5,5-dimethylhydantoin (DBH) is used as an oxidant resulting in a compound having a high yield of fluor atom incorporation.

9. A [^{18}F]-labelled compound obtainable by a method according to one of the claims 5, 6, 7 or 8.

10. A first intermediate compound having the general formula of an amino acid derivative which is N-protected by an imido group or a synthetically equivalent group and wherein the carboxyl function has been transformed into a dithioester function or a synthetically equivalent persulphurated moiety.

11. A first intermediate compound according to claim 10, wherein the imido group is a phthalimido group.

12. A first intermediate compound according to claim 10 or 11, obtainable via steps a to g of the method as claimed in claim 7.

13. A first intermediate compound according to claim 10, 11 or 12, being ethyl 3-(N-phthalimido)-aminopropanedithioate, N-(3,3,3-trifluoro-2-thioxopropyl) phthalimide, N-{[2-(trifluoromethyl)-1,3-dithiolan-2-yl] methyl} phthalimide, methyl (or ethyl) 3-phthalimide-2,2-difluoropropanedithioate, N-[2,2-difluoro-3,3,3-tris(methylthio) propyl] phthalimide or N-[2,2-difluoro-3,3,3-tris(ethylthio)propyl] phthalimide.

14. A second intermediate compound having the general formula of a [^{18}F]-labelled perfluorinated amino acid derivative which is N-protected by an imido group or a synthetically equivalent group.

15. A second intermediate compound according to claim 14, wherein the imido group is a phthalimido group.

16. A second intermediate compound according to claim 14 or 15, obtainable via steps a to h of the method as claimed in claim 7 or 8.

17. A second intermediate compound according to claim 14, 15 or 16, being N-(3,3,3-trifluoropropyl)phthalimide.

18. A third intermediate compound having the general formula of a [^{18}F]-labelled perfluoroalkyl amine.

19. A third intermediate compound according to claim 18, being [^{18}F]-labelled 3,3,3-trifluoropropyl amine.

20. A third intermediate [^{18}F]-labelled compound obtainable via steps a to i of the method as claimed in claim 7 or 8.

21. Use of a compound according to one of the claims 1-4 as bioactive compound.

22. A [^{18}F] labelled bioactive compound synthesized using as intermediates a first intermediate as claimed in one of the claims 10-13, a second intermediate as claimed in one of the claims 14-17 and a third intermediate as claimed in one of the claims 10-13.

23. A [^{18}F] labelled bioactive compound synthesized using as intermediates a first intermediate as claimed in one of the claims 10-13.

24. Method of perfuorination using as an intermediate a compound as claimed in one of the claims 10-13.

25. The compound of claim 22 which is an [^{18}F] labelled perfluorinated nitroimidazole compound having an incorporation of [^{18}F] atoms characterized by a specific radioactivity of the compound comprised between 1 and 30 Ci/mmol, preferably between 1 and 20 Ci/mmol, preferably 1 and 10 Ci/mmol.

26. A method for the detection of tissue hypoxia in a patient comprising:
- introducing an [^{18}F] labelled nitroimidazole compound of any of claims 1 to 4 into said patient,
- imaging tissue hypoxia in said patient, and
- quantifying tissue hypoxia in said patient.

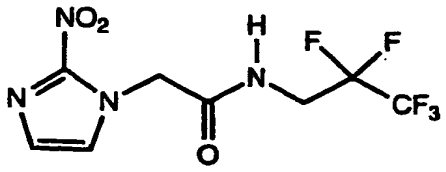
27. A method according to claim 26 wherein the detection technique used in said method is positron emission tomography.

28. A method for the detection of tissue hypoxia in a tissue comprising:
- introducing an [^{18}F] labelled nitroimidazole compound of any of claims 1 to 4 into a patient,
- removing a tissue sample from said patient, and
- analysing the emission in said tissue sample by autoradiography.

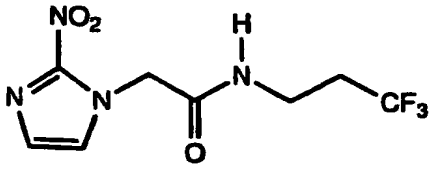
29. A method for the detection of an [^{18}F] labelled bioactive compound in a patient comprising:
- introducing an [^{18}F] labelled bioactive compound according to claim 1-4 into said patient,
- imaging the presence of said [^{18}F] labelled bioactive compound in said patient, and
- optionally, quantifying the presence of said [^{18}F] labelled bioactive compound in said patient.

30. A method for the detection of [^{18}F] labelled bioactive compound in a tissue comprising:

- introducing an [^{18}F] labelled bioactive compound of claim 1-4 into a patient,
- taking a tissue sample from said patient, and
- analysing the emission in said tissue sample by autoradiography.

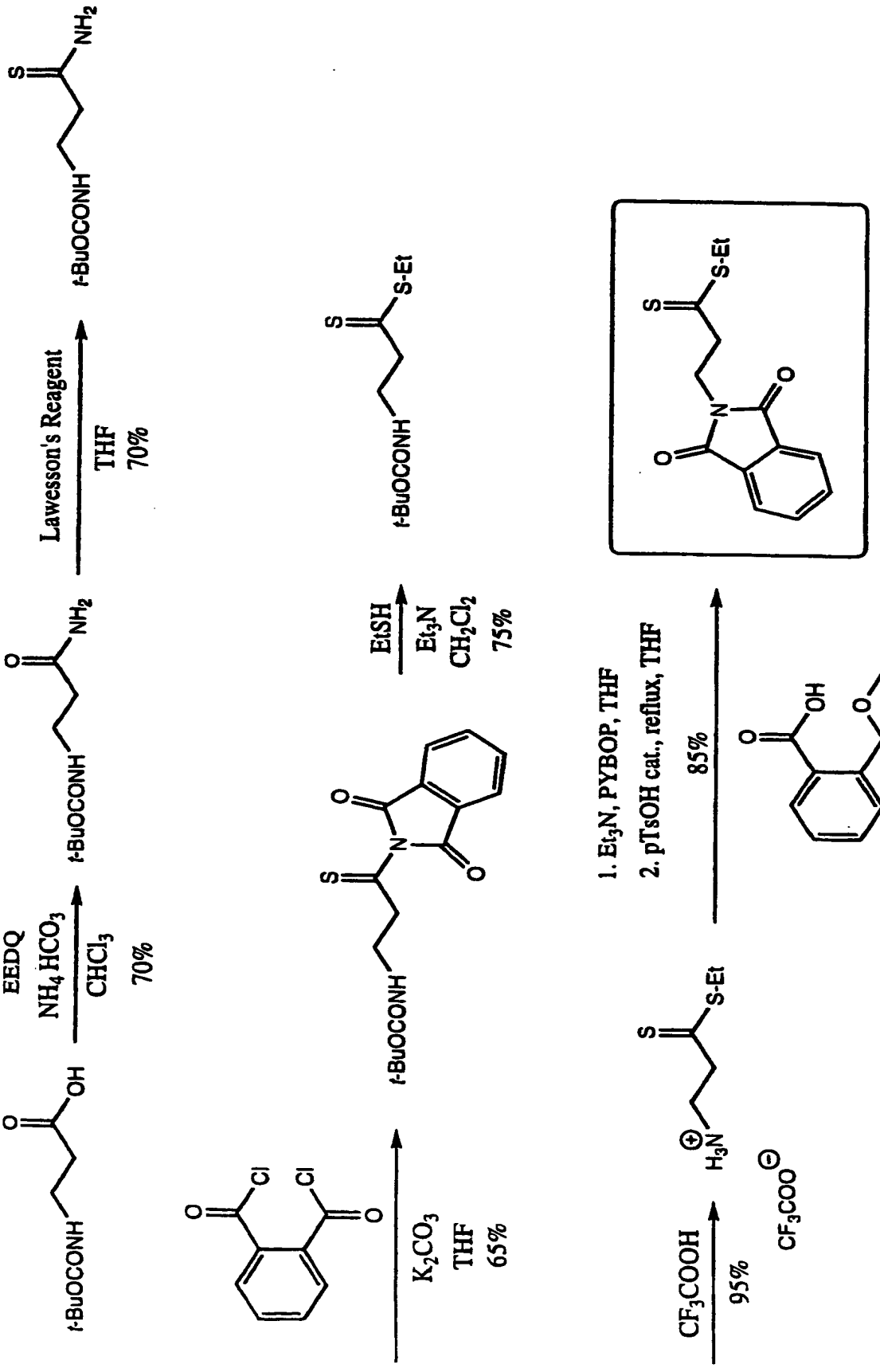


EF5



EF3

Figure 1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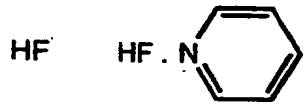
OH
EEDQ
NH4HCO3
CHCl3
70%
t-BuOCONH
NH2
Lawesson's Reagent
THF
70%
t-BuOCONH
NH2
S
K2CO3
THF
65%
t-BuOCONH
EtSH
Et3N
CH2Cl2
75%
t-BuOCONH
S-Et
S
1. Et3N, PYBOP, THF
2. pTsOH cat., reflux, THF
85%
CF3COOH
95%
H3N
CF3COO
OH
O

Figure 2

Pertluorinating agents

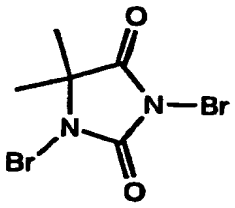


70% 30%

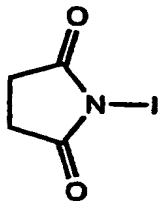
HF-Pyridine

$TBAH_2F_3$

Oxidants



DBH



NIS

Figure 3

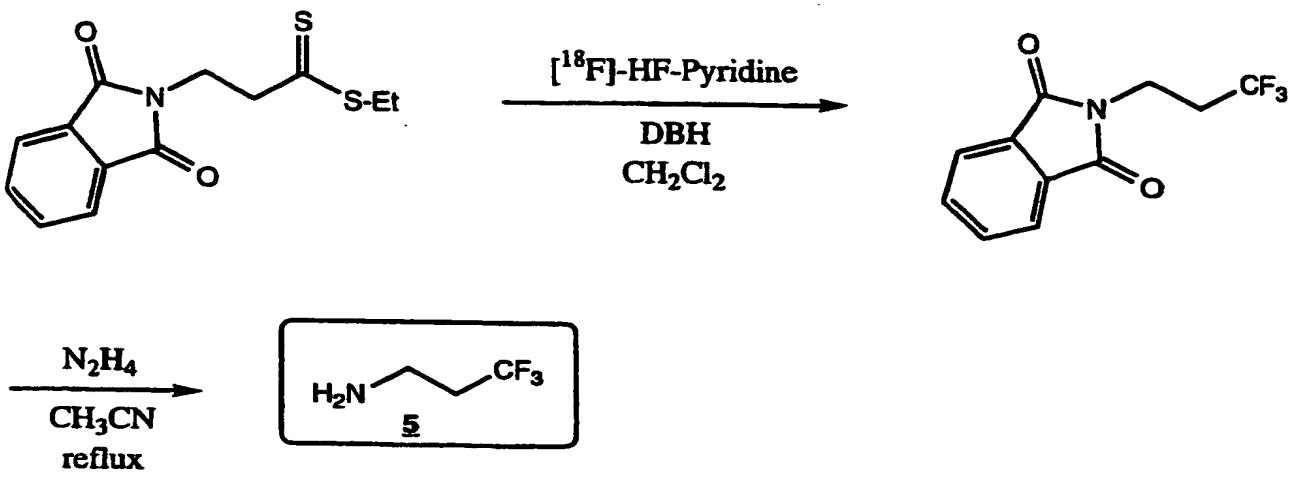

O
S
N
S-Et
O
[18F]-HF-Pyridine
DBH
CH2Cl2
O
N
CF3
O
N2H4
CH3CN
reflux
H2N
CF3
5

Figure 4

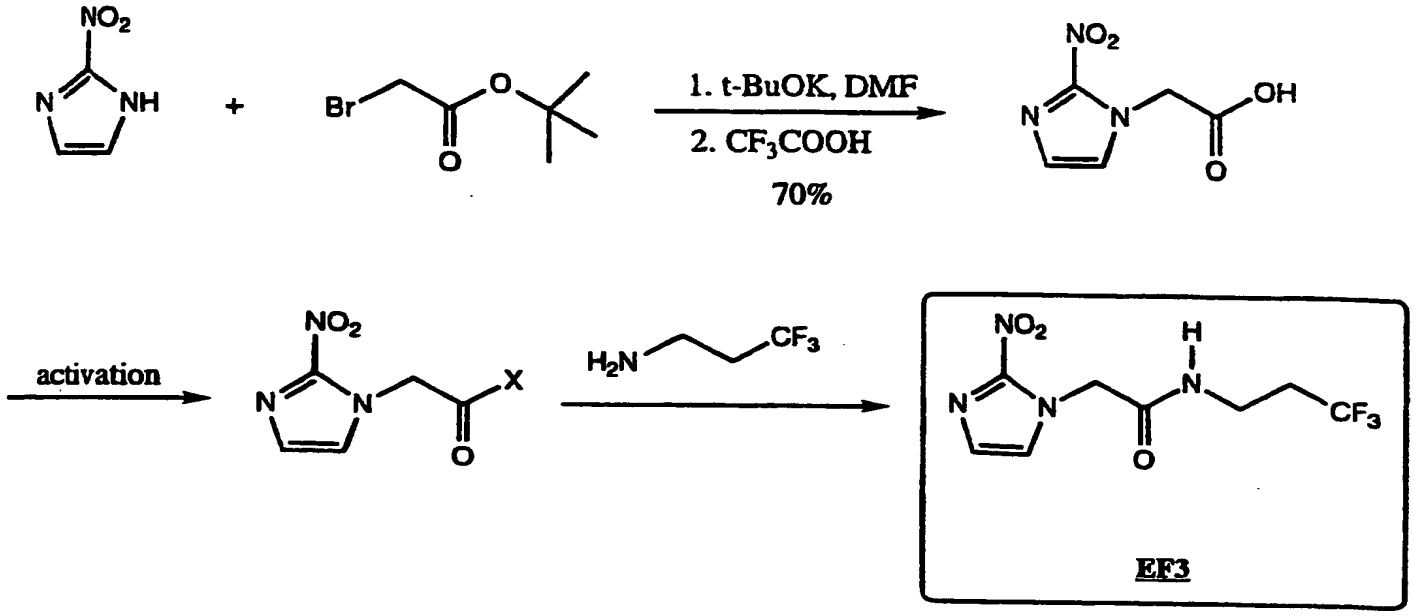

NO2
N
NH
+
Br
O
O
1. t-BuOK, DMF
2. CF3COOH
70%
NO2
N
N
OH
O
activation
NO2
N
N
X
O
H2N
CF3
NO2
N
N
H
N
O
CF3
EF3

Figure 5

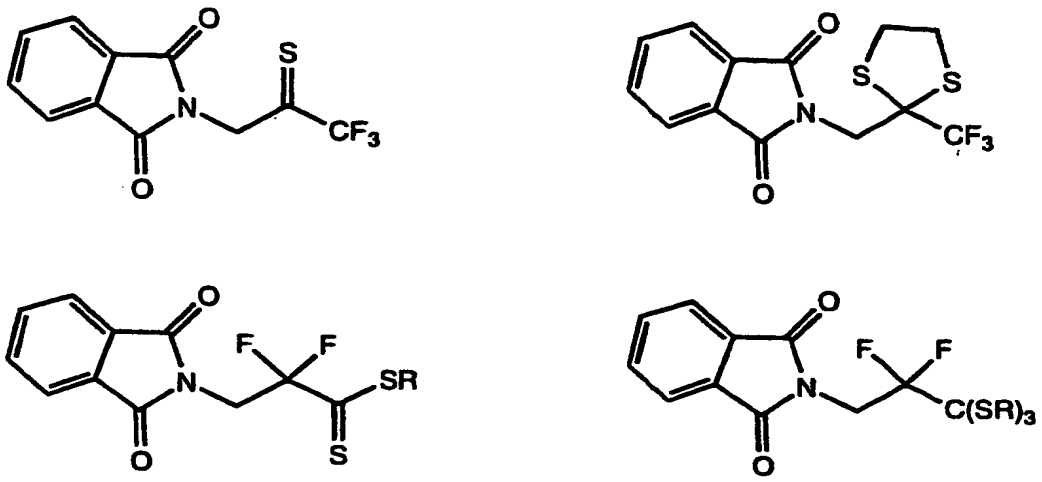


Figure 6

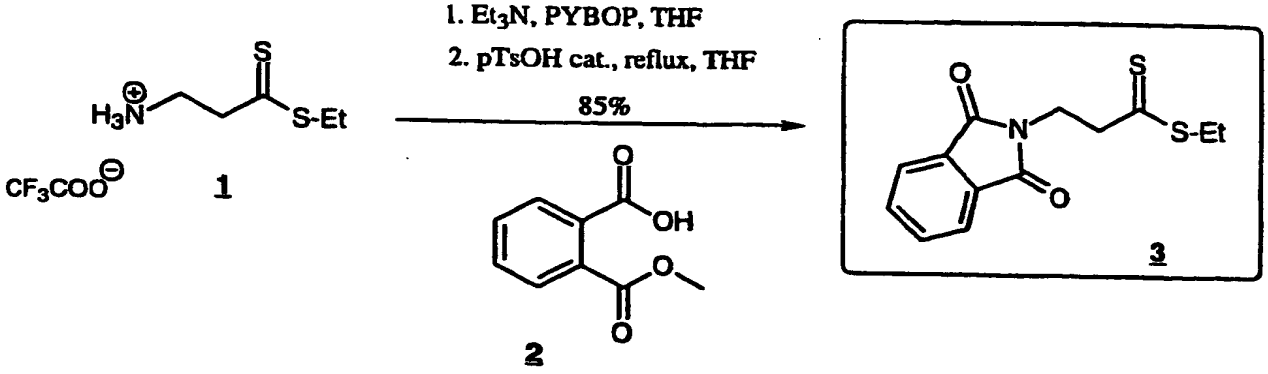


Figure 7

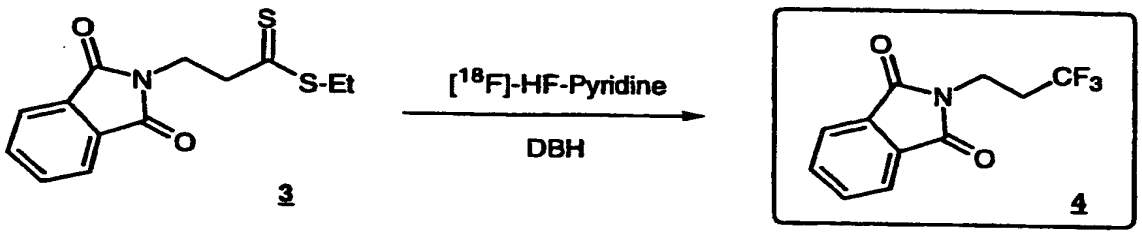


Figure 8

INTERNATIONAL SEARCH REPORT

Ir [redacted] l Application No
PCT/EP 00/04632

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07B59/00 C07D209/48 C07C211/03 G01N33/58

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07B C07D C07C G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
BEILSTEIN Data, CHEM ABS Data, WPI Data, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	J. B. DICKEY ET AL.: "Fluorinated Anthraquinone Dyes" INDUSTRIAL AND ENGENEERING CHEMISTRY, vol. 48, 1956, pages 209-213, XP000874162 WASHINGTON, US page 212, column 2, line 17 - line 37 ---	10
A	WO 94 11348 A (THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA) 26 May 1994 (1994-05-26) cited in the application page 212, column 2, line 17; claims; example 1 --- -/--	1,10, 21-30

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 September 2000	22/09/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Zervas, B

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/04632

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	OLIVIER JOSSE ET AL.: "A Convenient Synthesis of Ethyl 3-Aminopropanedithioate (beta-Alanine Ethyl Dithioester)" SYNTHESIS, March 1999 (1999-03), pages 404-406, XP002129104 STUTTGART DE cited in the application page 404, column 2; claims; example 1 page 405, column 2, line 7 - line 13; example 1 ---	10
A	WO 95 09844 A (BOARD OF RAGENTS, THE UNIVERSITY OF TEXAS SYSTEM) 13 April 1995 (1995-04-13) page 405, column 2, line 7; claims; examples -----	1,10, 21-30

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 00/04632

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9411348 A	26-05-1994	CA 2149770 A	26-05-1994
		EP 0669913 A	06-09-1995
		JP 8503469 T	16-04-1996
		US 5540908 A	30-07-1996
		US 5843404 A	01-12-1998
WO 9509844 A	13-04-1995	AU 8074294 A	01-05-1995
		US 5728843 A	17-03-1998
		US 5886190 A	23-03-1999